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Facilitation of cometabolic degradation of 4-chlorophenol using glucose as an added growth substrate

Si-Jing Wang & Kai-Chee Loh*
*Department of Chemical and Environmental Engineering, National University of Singapore, 10 Kent Ridge Crescent, S119260, Singapore (*author for correspondence)*

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Abstract

This paper reports on the feasibility of using glucose as an added substrate for cometabolic transformation of 4-chlorophenol (4-cp). When glucose was fed as the added growth substrate, only 78% and 43% of the initial 4-cp concentrations of 100 and 200 mg l^{-1}, respectively, were transformed before the pH dropped to below 4.5 and stopped all reactions. By maintaining the medium pH, complete removal of 4-cp was achieved even at the high initial concentration of 200 mg l^{-1}. Phenol induction prior to inoculation was not a prerequisite to ensure transformation of 4-cp when glucose was the added growth substrate. Compared with phenol as the added growth substrate, cells grown on glucose displayed a longer acclimation phase and, in general, a lower specific transformation rate. The volumetric transformation rate of 4-cp, however, was greatly enhanced due to the increased cell density. The results of this work suggest that 4-cp itself induced the enzymes necessary for its cometabolism. With NADH regenerated effectively through metabolism of glucose, 4-cp was transformed in the absence of added phenol. Consequently, the competitive inhibition involved in cometabolism was avoided and the risks associated with addition of toxic growth substrates such as phenol were eliminated.

Introduction

For biological degradation of toxic compounds degraded through cometabolic pathways, a suitable growth substrate, which serves as sources of carbon and energy to support cell growth, is required. Such dependence, however, is retarding the development of engineered biodegradation systems using cometabolizing microorganisms (Alexander 1994). It is quite common that an organic compound is chosen as a growth substrate because it can support cell growth of the cometabolizing bacterium naturally. For example, bacterium is isolated from polluted sites contaminated with this compound, in which case the bacterium is also found to be able to cometabolize the nongrowth substrate concerned (Chang et al. 1993; Little et al. 1988; Mu & Scow 1994). However, these "natural" growth substrates are usually environmentally toxic and the risks associated with the addition of such toxic chemicals can be high especially if the contaminated sites do not contain these "natural" growth substrates.

Besides supporting cell growth, growth substrates also serve to induce the enzymes and cofactors required for cometabolism. The enzymes involved in cometabolic reactions usually act on a series of closely related molecules and are not absolutely specific for a single substrate (Alexander 1994). Some even catalyze a single type of reactions on a variety of structurally dissimilar molecules. For example, many aerobic cometabolic reactions are catalyzed by nonspecific oxygenase enzymes that use O_2 as the co-substrate and NADH as the reduction source to oxidize growth substrates and nongrowth substrates (Chang & Alvarez-Cohen 1995; Delgado et al. 1992; Shields et al. 1991). In cases where growth and nongrowth substrates share the same key enzymes, competitive inhibition is commonly observed, and cometabolic transformation of the nongrowth substrates is strongly affected by the

presence of the growth substrates (Loh & Wang 1998; Speitel et al. 1993; Strand et al. 1990). To exclude such competitive inhibition, two-stage or sequencing reactor systems have been proposed to decouple cell growth and transformation of the nongrowth substrates (Alvarez-Cohen & McCarty 1991a; McFarland et al. 1992; Segar et al. 1995). Nevertheless, and in addition to the systems being inherently complex, the degradation activity of the cells in such systems for the nongrowth substrates cannot be maintained due to an absence of growth substrates (Alvarez-Cohen & McCarty 1991b; Segar et al. 1995; Strand et al. 1990).

Alternatively, the competitive inhibition between growth and nongrowth substrates can be avoided if different enzymes catalyze their respective transformations. This is possible if the cometabolic enzymes were induced by the nongrowth substrate. Then, the growth substrate is only responsible for cell growth and regeneration of necessary co-substrates like NADH or NADPH. This being the case, growth substrates can be optimally chosen from a wide range of carbon sources, including nontoxic, readily degradable organic compounds. Especially when the selected growth substrate is a conventional carbon source, the design of cometabolic systems can be facilitated with reduced cost and risks associated with the addition of toxic growth substrates can be eradicated.

This research investigates the hypothesis by using glucose as an added growth substrate for cometabolic transformation of 4-chlorophenol (4-cp). 4-cp was selected since it has been extensively studied as a nongrowth substrate by *Pseudomonas* species (Loh & Wang 1998; Sáez & Rittmann 1993, Schmidt et al. 1983, Spain & Gibson 1988). Glucose is a common conventional carbon source that has been widely used in biotransformation research (Hess et al. 1990; Papanastasiou & Maier 1982; Rozich & Colvin 1986; Wang et al. 1996). Although it has been reported that the cometabolic transformation rate of 4-cp in the presence of phenol can be enhanced by augmenting the medium with a conventional carbon source, such as sodium glutamate and glucose (Loh & Wang 1998), research on cometabolism of 4-cp with bacterial cells grown solely on a conventional carbon source like glucose is absent.

Materials and methods

The bacterium *Pseudomonas putida* ATCC 49451, which is able to grow on phenol and cometabolize 4-cp, was used throughout this work. Stock cultures of *P. putida* were maintained by periodic sub-transfer on nutrient agar (Oxoid, Hampshire, UK) slants, which were stored at 4 °C in the refrigerator. All batch cultures were grown with agitation at 200 rpm and at 30 °C in the mineral salt medium described by Loh & Wang (1998) in 500-ml Erlenmeyer flasks stoppered with a cotton plug at 50% medium volume. For preparation of inocula, cells from the nutrient agar slant were induced with the basal medium supplemented with 200 mg l^{-1} of phenol as the sole carbon source. For cases in which the effect of induction was investigated, the inocula were prepared by transferring cells maintained on agar slants into the medium containing 1 g l^{-1} of glucose as the only carbon source. Two ml of the phenol-induced (or glucose-grown) cells in the exponential growth phase were used for the biotransformation experiments.

For experiments of 4-cp biotransformation, the basal medium was supplemented with 1 g l^{-1} glucose (BHD analyzed Reagent, UK) as an added growth substrate. After inoculation, 4-cp was added directly from stock solution to give the desired initial concentrations. 4-cp (Merck, Darmstadt, Germany) was dissolved in 0.02 N NaOH solution to make 10,000 mg l^{-1} stock solution.

Samples were withdrawn periodically for analysis. Cell density, medium pH, and concentration of 4-cp were monitored. A 6 ml sample from each flask was taken for determination of pH and biomass. Cell density was monitored spectrophotometrically by measuring the absorbance at 600 nm. pH was measured using a small pH electrode and pH meter (Model HI 9021, Hanna Instruments, U.S.A.) suitable for small volume samples. For the analysis of 4-cp, a further 3 ml sample was taken and immediately acidified to pH 2 with 6N sulphuric acid to quench the biotransformation reaction. The acidified samples were extracted with 3 ml of methylene chloride (Merck, Darmstadt, Germany), which contained 100 mg l^{-1} *o*-cresol (Merck, Darmstadt, Germany) as an internal standard. The extract was analyzed for 4-cp concentration by gas chromatography (Perkin Elmer, Model 8700).

The average biotransformation rates were calculated by dividing the net amount of transformed 4-cp for the time period when the compound changed from 90% to 10% of the initial concentration (or the percentage which cannot be further degraded) by the corresponding elapsed time. To evaluate the degradation activity of the bacterial cells utilizing different growth

substrates, the specific transformation rate of 4-cp was calculated. Data for 4-cp concentration versus degradation time were correlated using the proper multivariable regression function in Microsoft Excel to obtain the equations of best fit of the degradation curves. The correlation, which reflects the real curvature of the degradation profile, was differentiated with respect to time and then divided by the cell mass at distinct times to determine the specific transformation rate of 4-cp. All analytical techniques and procedures have previously been described in detail (Lob & Wang 1998).

Results

In order to study the feasibility of using glucose as a growth substrate to cometabolize 4-cp, the biotransformation of 4-cp at two different concentrations, 100 mg l^{-1} and 200 mg l^{-1}, were carried out with 1 g l^{-1} glucose as the only added growth substrate. In addition, experiments were also performed to investigate whether phenol induction for the inoculum was critical for ensuring the degradation ability of the cells when the only added growth substrate was glucose.

Comparison of glucose and phenol as the added growth substrate

The experiment was conducted with medium containing 1 g l^{-1} glucose as a growth substrate to cometabolize 4-cp at initial concentrations of 100 or 200 mg l^{-1} 4-cp. Here, the reported experiments (F1 – 100 mg l^{-1} and S1 – 200 mg l^{-1} 4-cp) using phenol as the added growth substrate (Loh & Wang 1998) are used for comparison. Figure 1 shows the time profiles of concentration of 4-cp (initial nominal concentration of 100 mg l^{-1}), cell growth, and pH for the separate cases of glucose and phenol as the added growth substrates. Figure 1(a) shows that using phenol (F1) allowed complete removal of 4-cp within 14 hours and gave an average transformation rate of 45 mg l^{-1} h^{-1}. In contrast, glucose allowed slow transformation during the first twelve hours, after which the transformation rate increased for the next four hours. At about the 16th hour, the transformation rate diminished and eventually stopped, leaving about 22% of the initial 4-cp untransformed in the medium.

Figure 1(b) shows the cell growth and pH profiles. Glucose gave a lag phase of about 12 hours, longer than the lag of 6 hours in the case of phenol.

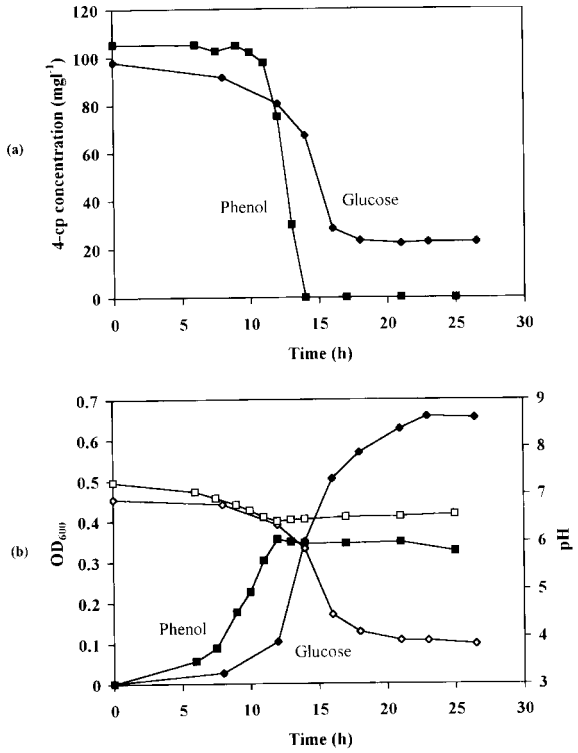

Figure 1. Comparison of glucose and phenol as the added growth substrate. (a) transformation of 100 mg l^{-1} 4-cp; (b) cell growth and pH profiles. Symbols: ♦, glucose as the growth substrate: ■, phenol as the growth substrate. The corresponding open symbols represent pH.

After the lag, the cells grew exponentially with a maximum specific growth rate of 0.38 h^{-1} using glucose and 0.35 h^{-1} using phenol. The highest cell density (OD_{600}) achieved was 0.65 using glucose. Figure 1(b) also shows that, with the fast cell growth on glucose, the rate of pH drop was also increased, and the pH quickly dipped below 4.5, consequently stopping further transformation of 4-cp.

Figure 2 plots 4-cp concentration, cell growth, and pH changes for the different added growth substrates at the higher 4-cp concentration of 200 mg l^{-1}. Glucose as the added growth substrate for cometabolic transformation of 200 mg l^{-1} 4-cp gave a slow transformation phase that extended for about 23 hours, compared to only 12 hours in the biotransformation of 100 mg l^{-1}. After this period, the transformation rate increased slightly. At the end of the experiment (32 hours from inoculation), about 57% of the initial 4-cp remained in the culture broth. The average transformation rate was only around 3 mg l^{-1} h^{-1}. In comparison, complete removal of 4-cp was achieved

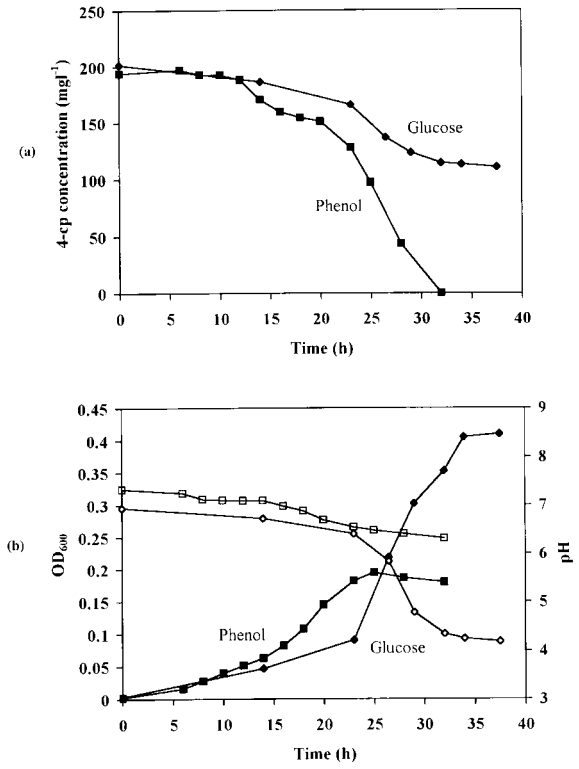

Figure 2. Comparison of glucose and phenol as the added growth substrate. (a) transformation of 200 mg l^{-1} 4-cp; (b) cell growth and pH time profiles. Symbols: ♦, glucose as the growth substrate; ■, phenol as the growth substrate. The corresponding open symbols represent pH.

within 32 hours when phenol was used as the added growth substrate, and the average transformation rate was 11 mg^{-1} l^{-1}.

Figure 2(b) shows that the lag phase for cells to grow on phenol was roughly one-half that needed when glucose was the added growth substrate. In both cases, the inoculating cells used were taken from the exponential phase of phenol-induced cells where the cell density (OD_{600}) was between 0.3 and 0.4. So the significant prolongation of the lag phase should be due to the type of growth substrate used, not a variation of the inoculum size or prior induction. The maximum specific growth rate on glucose was 0.2 h^{-1}, while that on phenol (S1), it was 0.13 h^{-1}. The highest cell density (OD_{600}) was achieved in glucose, 0.41 versus 0.20 when phenol was used. Similar to the biotransformation of 100 mg l^{-1} 4-cp (Figure 1(b)), pH was almost constant in the case of phenol as the growth substrate while the consumption of glucose resulted in a sharp pH drop. When pH dropped below 4.5, the transformation of 4-cp ceased. The next set of experiments involved a regulation of the medium pH so as to improve the biotransformation process.

Effect of pH regulation

In order to improve 4-cp transformation using glucose as the growth substrate, medium pH was regulated in two different experiments either by manual addition of 2N NaOH or *in-situ* by replacing the phosphate salt content of the medium with a phosphate buffer containing 1.74 g l^{-1} K_2HPO_4 and 0.24 g l^{-1} KH_2PO_4.

Figure 3 shows the biotransformation profiles for an initial concentration of 100 mg l^{-1} of 4-cp. The 4-cp was completely transformed after about 25 hours in both experiments. The average transformation rates were 9 mg l^{-1} h^{-1} for NaOH regulation and 11 mg l^{-1} h^{-1} for phosphate buffer regulation, respectively. Both of these rates still were much lower compared with using phenol as the added growth substrate, which was 45 mg l^{-1} h^{-1} for the case of 100 mg l^{-1} of 4-cp. Nevertheless, by regulating the pH of the medium, complete biotransformation of 100 mg l^{-1} of 4-cp was effected when the pH was kept between 6.5 and 7.5. The highest cell densities (OD_{600}) achieved were 0.72 (NaOH regulation) and 0.68 (buffer regulation), and the corresponding maximum specific growth rates were 0.35 h^{-1} and 0.33 h^{-1}.

Figure 4 shows 4-cp transformation at initial concentration of 200 mg l^{-1} using a phosphate buffer in the medium to maintain the pH. After about a 15 hour lag, the transformation rate increased rapidly, and complete transformation of 4-cp was achieved within 32 hours. The average transformation rate of 4-cp was 10 mg l^{-1} h^{-1}, almost as fast as that using phenol (11 mg l^{-1} h^{-1}) as the added growth substrate for the same concentration of 4-cp.

Based on the above results, it is apparent that the increase in initial concentration of 4-cp from 100 mg l^{-1} to 200 mg l^{-1} has no significant effect on the average 4-cp transformation rate when cells grew on glucose as the added growth substrate as long as the medium pH was regulated. However, the acclimation phase was significantly prolonged at 200 mg l^{-1} 4-cp. This can be attributed to inhibition of 4-cp towards cell growth. In fact, when the initial 4-cp concentration was increased to 300 mg l^{-1}, cells could not grow even after extended incubation (data not shown).

Figure 5 shows the specific transformation rate (STR) of 4-cp at initial 4-cp concentrations of 100 and 200 mg l^{-1} with glucose as the added growth sub-

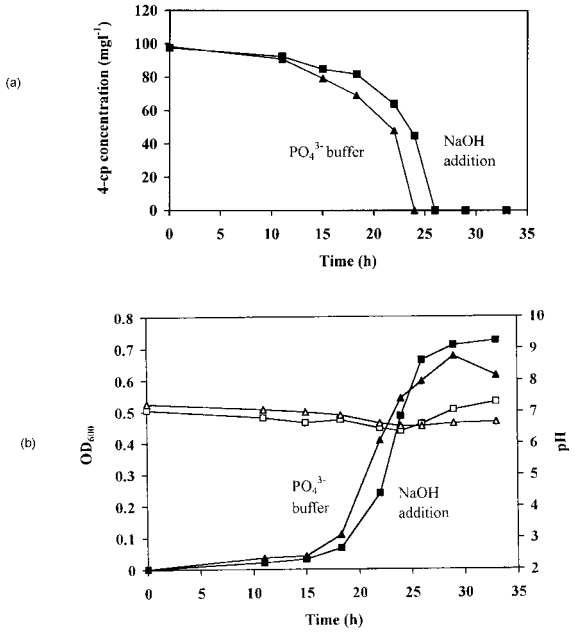

Figure 3. Effect of pH regulation on the transformation of 100 mg l^{-1} 4-cp with glucose as the added growth substrate. (a) transformation of 4-cp; (b) cell growth and pH profiles. Symbols: ■, using 2N NaOH; ▲, using phosphate buffer. The corresponding open symbols represent pH.

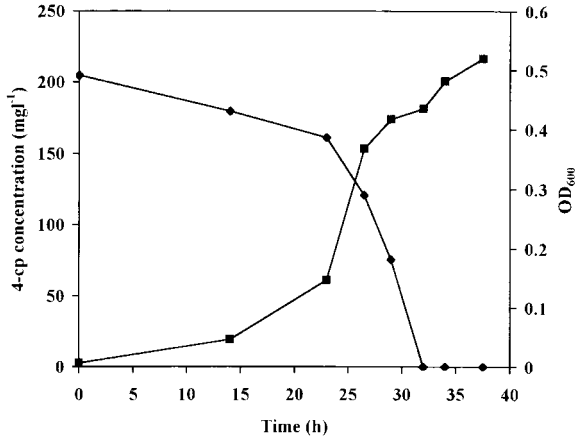

Figure 4. Transformation of 200 mg l^{-1} 4-cp using phosphate buffer to regulate medium pH with glucose as the added growth substrate. Symbols: ♦, 4-cp concentration; ■, biomass concentration.

strate. As reference, the 4-cp concentration profiles for the two experiments also are included in the figure. For the transformation of 100 mg l^{-1} 4-cp, the STR increased as biotransformation proceeded, and the STR was in the range of 28–45 mg(l.h.OD)$^{-1}$. Biotransformation of 4-cp at an initial concentration of 200 mg l^{-1} gave a similar upward pattern of the

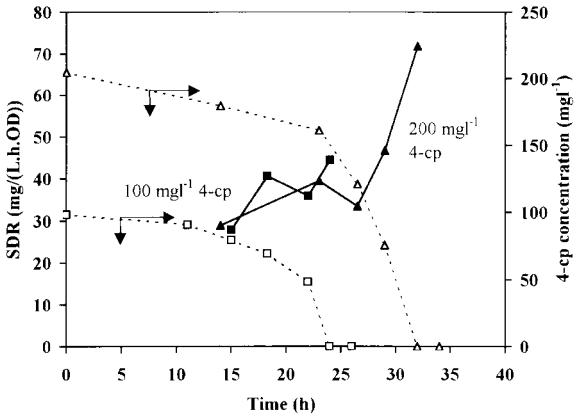

Figure 5. Time profiles of specific transformation rate of 4-cp using glucose as the added growth substrate under pH regulations. Symbols: ■, STR for initial 4-cp concentration of 100 mg l^{-1}; ▲, STR for initial 4-cp concentration of 200 mg l^{-1}. The corresponding open symbols represent 4-cp concentration.

STR. During the initial slow transformation period, the STR was within 30–40 mg/(l.h.OD), similar to that for transformation of 100 mg l^{-1} 4-cp. It subsequently increased significantly to 75 mg(l.h.OD)$^{-1}$ at the end of the transformation phase.

One possible interpretation of the increasing STR is that more cometabolic enzymes required for transformation of 4-cp were induced by 4-cp when cells were grown on glucose and without the addition of phenol.

Effect of phenol induction

To ascertain the need for phenol induction before inoculation, an experiment in which the inoculating cells were grown in 1 g l^{-1} glucose was performed. 2 ml of the glucose-grown culture were then used for inoculation to degrade 100 mg l^{-1} 4-cp with 1 g l^{-1} glucose as the added growth substrate. Since phenol induction may influence patterns of 4-cp transformation, cell growth, and pH, the medium pH was not regulated in this experiment in order to facilitate comparison with the respective experiment where phenol-induced inoculum was used. In addition, two "control" cultures with phenol-induced and glucose-raised inocula, respectively, were maintained with 4-cp and without glucose or phenol.

The transformation of 4-cp is depicted in Figure 6(a), and the cell growth and pH time profiles are presented in Figure 6(b). For the two "controls", no 4-cp transformation (Figure 6(a)) and no cell growth (Figure 6(b)) were observed. Figure 6(a) shows that

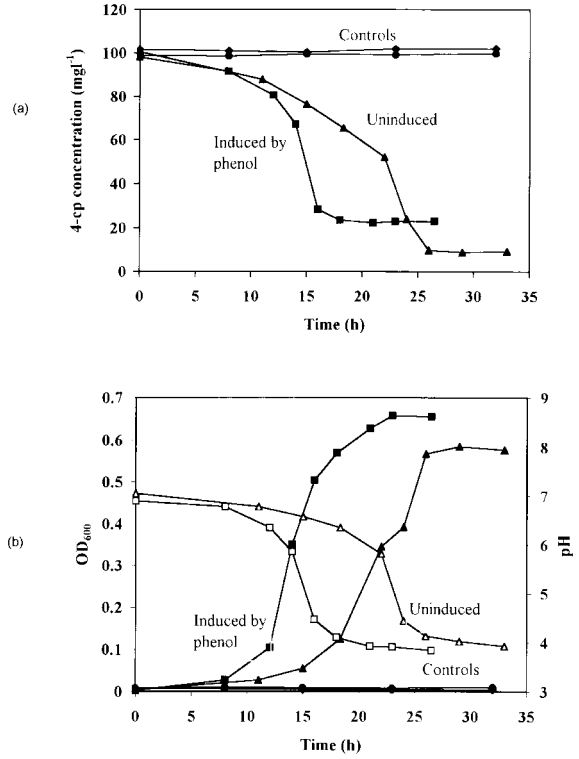

Figure 6. (a) Transformation of 4-cp using uninduced and induced inoculum. (b) Cell growth and pH using uninduced and induced inoculum. Symbols: ▲, ♦ (for "control") for uninduced inoculum; ■, ● (for "control") for induced inoculum. The corresponding open symbols represent pH.

the transformation of 4-cp, in the presence of glucose, exhibited the same trends whether the inoculum was phenol-induced or not, except for a shift in the curves due to a difference in the lag phase. After a relatively slow transformation phase, the transformation rate greatly increased for a short duration before coming to a drastic stop when the pH reached about 4.5. At the end of the experiments, 10–20% of the initial 4-cp remained untransformed in the culture medium, and the OD reached about 0.6.

Variations in the length of acclimation were observed in repeated experiments. In fact, a longer lag phase for phenol-induced cells was observed in some duplicate experiments of this study (data not shown). Many factors may affect the length of the acclimation such as the inoculum size, nature of the inoculum, the initial culture conditions, toxicity of 4-cp, and especially some undefined factors (Alexander 1994).

Despite variability, these results demonstrate that phenol induction before inoculation was not necessary for biotransformation of 4-cp. The results suggest again that 4-cp may induce phenol monooxygenase for transformation of 4-cp.

Discussion

4-cp is often reported to be cometabolized bn *Pseudomonas* species (Loh & Wang 1998. Sáez & Rittmann 1993, Spain & Gibson 1988). In such cases cells convert 4-cp initially to 4-chlorocatechol by an NADPH-dependent monooxygenase (Sáez & Rittmann 1991). 4-chlorocatechol is then further metabolized to a more polar compound (Spain & Gibson 1988), which has been suggested to be 2-hydroxy-5-chloromuconic semialdehyde (HCMSA) (Sáez & Rittmann 1991). Although often considered a dead-end product, the metabolite of the cometabolic transformation of 4-cp has been reported to be mineralized by a defined mixed culture (Schmidt et al. 1983). In cometabolic transformation of 4-cp, phenol is a good primary substrate since it can not only easily induce the monooxygenase required for 4-cp transformation, phenol oxidation can also efficiently regenerate consumed NADH. Strong competitive inhibition between phenol and 4-cp, however, inhibits 4-cp transformation significantly. It has been found that 4-cp was transformed rapidly only after phenol was almost fully depleted (Loh & Wang 1998). In addition, phenol is an environmentally toxic compound, the addition of which may result in additional pollution.

The results of this work show that 4-cp can be degraded in the absence of phenol by cells grown on glucose as the sole growth substrate. In this case, the cometabolic enzymes required for 4-cp transformation were most probably induced by 4-cp. This is likely since phenol and 4-cp are structurally analogous. Knackmuss and Hellwig (1978) have suggested that phenol monooxygenase in *Pseudomonas* sp. Strain B13 can be induced by either 4-cp or phenol. As for the cofactor NADH required for 4-cp transformation, this could be efficiently formed through the oxidation of glucose (Gottschalk 1979). After acclimation, with the rapid oxidation of glucose, NADH is quickly regenerated, consequently facilitating the transformation of 4-cp. As a result of using glucose as growth substrate, competitive inhibition with 4-cp can be avoided. Moreover, the use of glucose would not result in additional environmental pollution as opposed to using phenol.

The effect of growth substrate on 4-cp transformation can be further characterized by comparing the

specific transformation rates (STR) of 4-cp of cells grown on different growth substrates. Loh & Wang (1998) reported that, for transformation of 200 mg l^{-1} 4-cp with phenol as the added growth substrate, the STR for 4-cp decreased with phenol consumption from 112 mg(l.h.OD)$^{-1}$ to 32 mg(l.h.OD)$^{-1}$; upon depletion of phenol in the medium, the STR of 4-cp increased to 109 mg(l.h.OD)$^{-1}$. These clearly indicate the strong competitive inhibition between phenol and 4-cp. In contrast, we found that the STR of 4-cp gradually increased to 71 mg(l.h.OD)$^{-1}$ when glucose was the added substrate.

One possible problem of using glucose as an added growth substrate is an extended lag period. The problem of this long lag can be minimized if a continuous degradation system is employed.

The difference in the lag phase behavior when cells were exposed to different growth substrates can be explained based on the two types of lag phase – apparent lag and acclimation lag (Kim & Maier 1986). In the presence of phenol, for both 100 and 200 mg l^{-1} 4-cp, cells grew exponentially only after about 4–6 hours. This occurred because *P. putida* ATCC 49451 is characteristically known to be able to degrade phenol, and phenol can easily initiate enzymes necessary for its own degradation. In addition, cell activation in phenol-containing medium was performed before inoculation. Furthermore, since 4-cp is a structural analogue of phenol, cells did not need to "retool" their enzyme system. As a result, a short lag time was experienced. This lag phase is termed an "apparent lag" (Kim & Maier 1986). This observation is consistent with the literature that acclimation of a microbial community to one substrate frequently results in the simultaneous acclimation to some structurally related molecules since individual species often act on several structural analogues (Alexander 1994). For example, Shimp & Pfaender (1987) found that acclimation of the microbial community to phenol as a sole carbon source led to an increase in the ability of the organisms to degrade the structurally related aromatic compounds, such as 4-chlorophenol, *m*-aminophenol and *m*-cresol. On the other hand, when glucose was used as the sole growth substrate instead of phenol, prior to the exponential growth phase, both cell growth and 4-cp transformation were still observed, albeit very slowly. This period of slow transformation of 4-cp and slow growth observed can be attributed to what is termed "acclimation lag". This refers to the time for physiological changes in the metabolic system of cells to occur in response to exposure to a new environment. The classical explanation of this type of lag involves changes in regulation and production of enzymes and may involve changes in cell size and composition as well as changes in genetic characteristics (Alexander 1994; Kim & Maier 1986). When glucose was fed as the sole growth substrate, the metabolic system of the cells had to be changed in order to oxidize glucose in the presence of 4-cp. The activation and regulation of a specific enzyme system for this purpose might be a gradual process, during which both the rates of cell growth and 4-cp transformation were very slow. After the enzyme system was fully adapted to overcome the toxicity of 4-cp, cells grew quickly, and cofactors like NADH required for 4-cp transformation were generated rapidly, with concomitant increase in the 4-cp transformation rate.

Reports in the literature on cometabolism of environmentally hazardous compounds facilitated solely by a nontoxic, conventional carbon source have been rather limited. Fedorak and Grbic-Galic (1991) reported that glucose or peptone can replace 1-methylnaphthalene as growth substrate in an investigation of the biotransformation of benzothiophene and 3-methylbenzothiophene by a 1-methylnaphthalene-degrading enrichment culture. In another study, Cooney and Shiaris (1982) observed that several growth substrates, including yeast extract together with peptone, glucose, benzoate, and non-detergent motor oil together with kerosene, can support the cometabolism of phenanthrene by a microcosm of estuarine microorganisms. However, as these studies were performed with mixed cultures, direct consequence of the alternate growth substrate could not be ascertained since incubations with the new growth substrate such as glucose or peptone could have altered the composition of the culture population and also the composition of the culture broth. On the contrary, a *Pseudomonas* sp. Strain JS6 which grew on *p*-dichlorobenzene (*p*-DCB) and cometabolized 2,5-dichlorophenol (2,5-DCP) could not transform 2.5-DCP when cells were grown on glucose without *p*-DCB (Spain & Gibson 1988). In these studies, however, no further investigation was performed to elucidate the role of the conventional carbon sources like glucose.

Based on our study, several factors should be considered: (1) whether the cometabolic enzymes can be induced by the targeted compound (even by the conventional growth substrate) or they are constitutive; (2) whether some required cofactors like NADH or NADPH can be effectively generated by the oxida-

tion of the growth substrate; (3) the toxicity of the targeted compound and its inhibition on cell growth; and (4) culture environmental conditions like pH and catabolite or product inhibition.

In cometabolism, it is almost a general phenomenon that competitive inhibition between growth and nongrowth substrates occurs, since the same key enzymes are responsible for the transformation of growth and cometabolic substrates. Two-stage or sequencing reactor systems have been proposed to overcome the competitive inhibition. In this paper, an alternative solution was investigated. 4-cp was apparently responsible for the induction of enzymes required for its transformation. Cell growth and generation of cofactors like NADH were provided by metabolism of glucose, which avoided any competitive inhibition. The toxic growth substrate phenol was not needed. Further investigation on kinetics is needed for additional understanding of the system, as well as to rationalize process design and optimize operating parameters.

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References

Alexander M (1994) Biodegradation and Bioremediation. Academic Press, San Diego, California

Alvarez-Cohen L & McCarty PL (1991a) Two-stage dispersed-growth treatment of halogenated aliphatic compounds by cometabolism. Environ. Sci. Technol. 25: 1387–1393

Alvarez-Cohen L & McCarty PL (1991b) Product toxicity and cometabolic competitive inhibition modeling of chloroform and trichloroethylene transformation by methanotrophic resting cells. Appl. Environ. Microbiol. 57: 1031–1037.

Chang H-L & Alvarez-Cohen L (1995) Transformation capacities of chlorinated organics by mixed cultures enriched on methane, propane, toluene, or phenol. Biotechnol. Bioeng. 45: 440–449

Chang MK, Voice TC & Criddle CS (1993) Kinetics of competitive inhibition and cometabolism in the biodegradation of benzene, toluene, and *p*-xylene by two *Pseudomonas* isolates. Biotechnol. Bioeng. 41: 1057–1065

Cooney JJ & Shiaris MP (1982) Utilization and co-oxidation of aromatic hydrocarbons by estuarine microorganisms. Dev. Ind. Microbiol. 23: 177–185

Delgado A, Wubbolts MG, Abril MA, & Ramos JL (1992) Nitroaromatics are substrates for the TOL plasmid upper-pathway enzymes. Appl. Environ. Microbiol. 58: 415–417

Fedorak PM & Grbic-Galic D (1991) Aerobic microbial cometabolism of benzothiophene and 3-methylbenzothiophene. Appl. Environ. Microbiol. 57: 932–940

Gottschalk G (1979) Bacterial Metabolism. Springer-Verlag, Inc., New York

Hess TF, Schmidt SK, Silverstein J & Howe B (1990) Supplemental substrate enhancement of 2,4-dinitrophenol mineralization by a bacterial consortium. Appl. Environ Microbiol. 56: 1551–1558

Kim CJ & Maier WJ (1986) Acclimation and biodegradation of chlorinated organic compounds in the presence of alternate substrates. J. Water Pollut. Control Fed. 58: 157–164

Knackmuss H-J & Hellwig M (1978) Utilization and cooxidation of chlorinated phenols by *Pseudomonas* sp. B13. Arch. Microbiol. 117: 1–7

Little CD, Palumbo AV, Herbes SE, Lidstrom ME, Tyndall RL & Gilmer PJ (1988) Trichloroethylene biodegradation by a methane-oxidizing bacterium. Appl. Environ. Microbiol. 54: 951–956

Loh K-C & Wang S-J (1998) Enhancement of biodegradation of phenol and a nongrowth substrate 4-chlorophenol by medium augmentation with conventional carbon sources. Biodegradation 8: 329–338

McFarland MJ, Vogel CM & Spain JC (1992) Methanotrophic cometabolism of trichloroethylene (TCE) in a two-stage bioreactor system. Water Res. 26: 259–265

Mu DY & Scow KM (1994) Effect of trichloroethylene (TCE) and toluene concentrations on TCE and toluene biodegradation and the population density of TCE and toluene degraders in soil. Appl. Environ. Microbiol. 60: 2661–2665

Papanastasiou AC & Maier WJ (1982) Kinetics of biodegradation of 2,4-dichlorophenoxyacetate in the presence of glucose. Biotechnol. Bioeng. 24: 2001–2011

Rozich AF & Colvin RJ (1986) Effects of glucose on phenol biodegradation by heterogeneous populations. Biotechnol. Bioeng. 28: 965–971

Sáez PB & Rittmann BE (1991) Biodegradation kinetics of 4-chlorophenol, an inhibitory co-metabolite. Res. J. Water Pollut. Control Fed. 63: 838–847

Sáez PB & Rittmann BE (1991) Biodegradation kinetics of a mixture containing a primary substrate (phenol) and an inhibitory co-metabolite (4-chlorophenol). Biodegradation. 4: 3–21

Schmidt E, Hellwig M & Knackmuss HJ (1983) Degradation of chlorophenols by a defined mixed microbial community. Appl. Environ. Microbiol. 46: 1038–1044

Segar Jr. RL, De Wys SL & Speitel Jr. GE (1995) Sustained trichloroethylene cometabolism by phenol-degrading bacteria in sequencing biofilm reactors. Water Environ. Res. 67: 764–774

Shields MS, Montgomery SO, Cuskey SM, Chapman PJ & Pritchard PH (1991) Mutants of *Pseudomonas cepacia* G4 defective in catabolism of aromatic compounds and trichloroethylene. Appl. Environ. Microbiol. 57: 1935–1941

Shimp RJ & Pfaender FK (1987) Effect of adaptation to phenol on biodegradation of monosubstituted phenols by aquatic microbial communities. Appl. Environ. Microbiol. 53: 1496–1499

Spain JC & Gibson DT (1988) Oxidation of substituted phenols by *Pseudomonas putida* F1 and *Pseudomonas* sp. Strain JS6. Appl. Environ. Microbiol. 54: 1399–1404

Speitel Jr. GE, Thompson RC & Weissman D (1993) Biodegradation kinetics of *Methylosinus trichosporium* OB3b at low concentrations of chloroform in the presence and absence of enzyme competition by methane. Water Res. 27: 15–24

Strand SE, Bjelland MD & Stensel HD (1990) Kinetics of chlorinated hydrocarbon degradation by suspended cultures of methane-oxidizing bacteria. Res. J. Water Pollut. Control Fed. 62: 124–129

Wang K-W, Baltzis BC & Lewandowski GA (1996) Kinetics of phenol biodegradation in the presence of glucose. Biotechnol. Bioeng. 51: 87–94